Clin. exp. Immunol. (1982) **48,** 375–380.

Pertussis vaccine inhibits immune insulitis induced with streptozotocin

SHIH-WEN HUANG & G.E. TAYLOR *Division of Pediatric Allergy and Immunology, Department of Pediatrics, University of Maryland, School of Medicine, Baltimore, Maryland, USA*

(Accepted for publication 4 December 1981)

SUMMARY

Normally, the injection of streptozotocin (STZ) at a lower dose (60 mg/kg body weight) to young (45 days old) CD-1 male mice produces a sustained hyperglycemia with the concomitant development of hypoinsulinemia and immune insulitis in the pancreas, both of which lead to insulin-dependent diabetes (IDD). In an effort to abort the development of IDD, pertussis vaccine (PV) was administered either intraperitoneally ($n=12$) or intravenously ($n=12$) 3 days prior to STZ injection. In contrast to the control group ($n=12$) which received only STZ resulting in the subsequent development of IDD after 16 weeks, none of the vaccinated group developed IDD. The complete protective effect was evidenced by normal insulin values, normoglycemia, the lack of the development of nucleic acid antibody and the absence of insulitis in the vaccinated animals. Under these experimental conditions, PV appeared to offer satisfactory protection of the β cells of islets in pancreas against the inflammatory effect of STZ.

INTRODUCTION

Streptozotocin (STZ), even when administered in a low dose, may produce a permanent form of insulin-dependent diabetes (IDD) in experimental animals (Like & Rossini, 1976; Like *et al.*, 1978; Rossini *et al.*, 1978; Maclaren *et al.*, 1980; Huang & Taylor, 1981). This STZ-induced diabetes has become an increasingly useful model to study the pathogenesis of recent onset IDD in man primarily because it approximates clinical findings (Gepts & DeMey, 1978; Like & Rossini, 1976; Like *et al.*, 1978; Huang & Taylor, 1981) and also because it lends itself easily to controlled manipulation. Several attempts have been made to restrict or prevent the development of diabetes. For example, anti-lymphocyte sera was found to be effective in preventing this form of diabetes (Rossini *et al.*, 1978), thus supporting the assertion that immunocytes participate in the development of the disease. It was also observed that pertussis vaccine (PV) appears to curtail STZ-induced diabetes (Katada & Ui, 1977), but a correlation between this protection and immunological and histological findings in such vaccinated mice has not been elucidated. The present report describes biochemical, histological and immunological changes associated with the onset of STZ-induced diabetes and its reversal by PV and provides practical and theoretical significance for intervening in the insulitis process.

MATERIALS AND METHODS

Animals. CD-1 male mice (Charles River Laboratory, Wilmington, Massachusetts, USA) were

Correspondence: Dr Shih-Wen Huang, Department of Pediatrics, University of Maryland Hospital, 22 S. Greene Street, Baltimore, Maryland 21201, USA.

0009-9104/82/0500-0375$02.00

obtained at the age of 35 days. The mice were kept in animal quarters for 10 days before the experiment. Laboratory chow and water was given *ad libitum*. Mice were weighed before and during the experiments on a weekly basis. They were divided into three groups—control and two groups receiving different doses of PV by different routes.

Streptozotocin (STZ). STZ (a gift from Dr Paul W. O'Connell from the Upjohn Co., Kalamazoo, Michigan; Lot No. 60, 273-2) was dissolved in citrate buffer, pH 4·2, immediately before experiment (Huang & Taylor, 1981). Each mouse received single intraperitoneal injection of STZ at a dose of 60 mg/kg body weight (in the amount of $\leq 0{\cdot}5$ ml) on 0 day.

Pertussis vaccine (PV). PV (a gift from Dr Charles R. Manclark of Bureau of Biologics, FDA, Rockville, Maryland, USA, Lot 7b) in the form of whole-cell *Bordetella pertussis* vaccine was given to the two groups of mice ($n=12$ each) 3 days prior to STZ injection. One group received a single intraperitoneal (i.p.) injection of 0·25 ml PV containing approximately $1{\cdot}2 \times 10^{10}$ organisms; the other group received a single intravenous (i.v.) injection of 0·25 ml PV containing approximately $0{\cdot}6 \times 10^{10}$ organisms. The control group ($n=12$) received intraperitoneally 0·25 ml of 0·01% merthiolate solution which was used as a diluent for the vaccine.

Blood glucose. Duplicate blood samples in 20 μl heparin-treated pipettes) were obtained from the paraorbital venous plexus. Blood glucose was measured by the method published previously (Mark, 1959) in all three groups on the following schedule: day -3 and day 0 relative to STZ injection and weekly thereafter.

Glucose tolerance test (TTT). A glucose solution (10% solution, 1·0 ml/100 gm body wt) was injected i.p. into mice fasted for 20 hr and blood samples were taken for analysis 15, 30, 60, 90, 120 and 180 min thereafter. The test was performed on day 60 in mice which received STZ only. In mice that received PV together with STZ (on day -3), either i.p. or i.v., as well as in mice which received neither PV nor STZ, the test was performed on day 70.

Insulin assay. Except in some diabetic mice, plasma insulin was measured at the end of the experiments by radioimmunoassay (BIO-RIA Inc., Louisville, Kentucky, USA).

Antibody to nucleic acids. The antibodies to nucleic acids were detected by passive microhaemagglutination technique as described previously (Huang & Maclaren, 1978). The antigens for the study included synthetic double-stranded RNA (Poly A·Poly U and Poly I·Poly C) and single-stranded DNA of calf thymus (Sigma Chemical, St Louis, Missouri, USA). The antibodies were determined monthly.

Histological examination of pancreas. The staining of the tissues and the histological examination of the islets in pancreas for each mouse was described previously (Huang & Taylor, 1981). For diabetic mice, pancreas were removed when their blood glucose levels reached and remained for at least one week above 400 mg%. For non-diabetic mice, the pancreas were examined 16 weeks after STZ injection.

Statistical analysis. Chi-square analysis on *t*-Test for small samples was performed to compare the findings among groups of mice.

RESULTS

Fig. 1 illustrates the time course of blood glucose levels of mice injected with STZ only or with STZ and PV. All mice receiving STZ alone developed persistent hyperglycemia (blood glucose above 150 mg%) between 4 and 6 weeks after injection and they were sacrificed around 10 weeks when their hyperglycemia became irreversible. In contrast, mice which received PV either i.v. or i.p. did not develop hyperglycemia. Within the first 7 days after PV injection, mice uniformly developed transient hypoglycemia (blood glucose lower than 50 mg%), but they returned to a normoglycemic state (blood glucose between 50 and 150 mg%) which lasted for at least 16 weeks after STZ injection (Fig. 1).

The results of the glucose tolerance test are illustrated in Fig. 2 where the mice that received only STZ showed a curve that was significantly abnormal. In contrast, the mice treated with both PV (either i.v. or i.p.) and STZ showed a normal course of blood glucose changes, similar to untreated

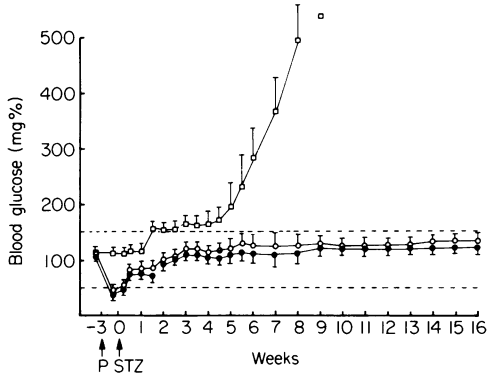

Fig. 1. Sequential changes of blood glucose levels in three groups of mice ($n = 12$ each) receiving PV and/or STV. PV was administered i.p. ($1{\cdot}2 \times 10^{10}$ organisms; ●——●) or i.v. ($0{\cdot}6 \times 10^{10}$ organisms; ○——○) 3 days prior to STZ injection (60 mg/ml body wt.). Control mice □——□) received only STZ. The vertical bars indicate one standard deviation from the mean values. Normal range of blood glucose levels is expressed by two broken lines.

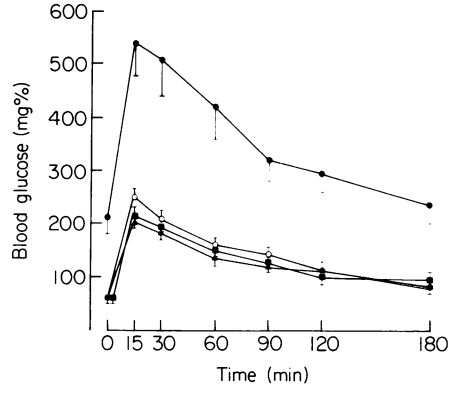

Fig. 2. The results of glucose tolerance test in four groups of mice ($n = 5$ each): (1) group received STZ only (●——●), (2) group received PV i.p. ($1{\cdot}2 \times 10^{10}$ organisms) 3 days before STZ (■——■), (3) group received PV i.v. ($0{\cdot}6 \times 10^{10}$ organisms) 3 days before STZ (○——○) and (4) group received no PV nor STZ (▲——▲). The test was performed on day 40 for the group (1) and day 70 for the remaining groups. The vertical bars indicate one standard deviation from the mean values.

Table 1. Plasma insulin and histological findings

Group	*n*	Insulin (μu/ml) (mean ± sem)	No. of mice showing	
			Insulitis	Change in size of islets
(1) STZ+PV (i.v.)	12	52·8 ± 1·8 (112)*	0	0
(2) STZ+PV (i.p.)	12	53·5 ± 1·4 (112)	0	0
(3) STZ only	12	11·0 ± 2·4 (60)	7	12
(4) Control (no PV or STZ)	12	57·0 ± 1·0 (112)	0	0
P†		<0·01	<0·01	<0·01

( )* indicates the days after STZ inj.

†*P* value based on the statistical difference between group (3) and group (1), (2), (4), respectively

Table 2. The incidence of antibodies against various nucleic acid antigens in mice received either STZ, STZ plus PV and in control mice.

Group	*n*	Poly A·Poly U	Poly I·Poly C	ssDNA
(1) STZ+PV (i.v.)	12	0 (0·0)	0 (0·0)	0 (0·0)
(2) STZ+PV (i.p.)	12	1 (8·3)	0 (0·0)	1 (8·3)
(3) STZ only	12	9 (75·0)	5 (41·7)	8 (66·7)
(4) Control (no PV or STZ)	12	0 (0·0)	0 (0·0)	0 (0·0)
*P**		<0·05	<0·05	<0·05

**P* values based on the statistical difference of the incidence between the mice in group (3) and group (1), (2), (4), respectively.

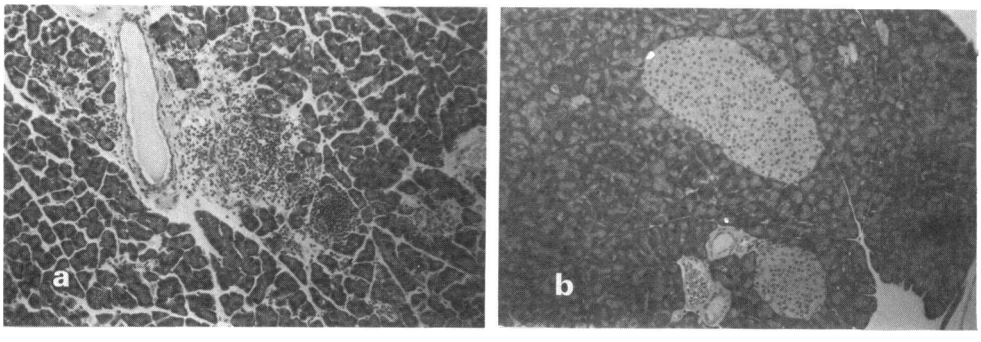

Fig. 3. (a) Illustration of insulitis in the pancreas from a mouse that received STZ alone. There are heavy infiltration of mononuclear cells in the islets. The tissue was obtained on day 40 after STZ injection at the time blood glucose level was 420 mg %. (b) Showing the appearance of islets from a mouse that received PV 3 days prior to STZ injection (i.p.). The tissue was obtained on day 112. Those islets appeared to be normal both in size and morphology.

mice. The difference in blood glucose levels between the group which received only STZ and others were statistically significant ($P < 0{\cdot}01$, Fig. 2).

The values of plasma insulin levels and the findings of histological changes between the groups are summarized in Table 1. The incidence of serum antibody against various nucleic acids are listed in Table 2.

Fig. 3 illustrates histological changes found in the pancreas of one animal receiving STZ only (3a) and one which received STZ and PV (i.p.) (3b). Such changes were typical and observed routinely.

The changes of the weight between the groups of mice throughout the experiments were not statistically significant.

DISCUSSION

Since STZ-induced insulitis in mice has become an acceptable model of experimentally induced diabetes, attempts to intervene immunologically in the development of the disease has been made. A combination of 3-*O*-methyl-*D*-glucose (3-OMG) and anti-lymphocyte serum resulted in a total prevention of hyperglycemia (Rossini *et al.*, 1978). Normal GTT values and normal histology of the pancreas support the assertion that 3-OMG and ALS are effective in abrogating the development of IDD if they were given timely. Recently, other immunological intervention including immunizing

animals with pertussis vaccine (PV) or giving hydrocortisone 3 days after STZ injection all reported success in reversing the hyperglycemic condition back to a normoglycemic state during a short course of observation (Kadada & Ui, 1977).

Our study was intended to further investigate the extent to which PV was effective in restricting STZ-induced diabetes (Huang & Taylor, 1981) over longer terms and to correlate the observed protection with a histological and biochemical return to normalcy. It was observed that PV administered, either by i.p. or i.v. route 3 days prior to STZ produced identical results even though the dose used intravenously was only half of that used intraperitoneally. Changes in blood glucose levels were almost identical between these two groups (Fig. 1). Complete protection against diabetes in these mice were substantiated by: (1) normal value of GTT, (2) normal plasma insulin values, (3) absence of antibody to nucleic acids, and (4) normal appearance of islets in the pancreas without changes of insulitis.

The mechanism of the protective effect of PV against IDD is still unknown but the response to some of the biological properties of PV should be considered. The endotoxin of *Bordetella pertussis* is known to possess all the usual biological components of enterobacterial endotoxins (Chaby *et al.*, 1979) and may act as an immunoregulator by altering the immune response in the host (Dresser & Phillips, 1973). Another biological factor, lymphocytosis-promoting factor (LPF) of *B. pertussis* (Munoz & Bergman, 1979) may also participate in suppressing insulitis via its unique effect on lymphocytes (Andersen *et al.*, 1979). Indeed, modulation of T cell proliferation by helper and suppressor lymphocytes was recently reported in the study of *in vitro* effects of *B. pertussis* (Ho, Kong & Morse, 1980). Finally, another biological factor of *B. pertussis*, islet-activating protein (IAP) (Ui *et al.*, 1977), by being a powerful adrenergic beta-receptor agonist on the cell membrane of β cell in islet (Sumi & Ui, 1975) may serve as an effective deterrent against the damage caused by STZ. As judged from our observation on the sequential changes of both lymphocytes and granulocytes and the development of splenomegaly (Huang & Taylor, unpublished) after PV immunization, it is most likely that the induced immunosuppressive effect in the vaccinated animals was the result of the combined effect of endotoxin and other biological factors.

Two points of interest worth mentioning are: (1) infectious agents, such as *B. pertussis* with its powerful biological effect, may sometimes serve as a natural safeguard against a disease such as IDD even for a genetically susceptible host, and (2) it may still be possible to interrupt the disease in its early stage if immune insulitis is primarily responsible for the islet cell destruction. Further study in this area should not only aid us in the better understanding of IDD, but also would raise possibilities of new strategy for more efficient management of the disease.

REFERENCES

ANDERSEN, V., HERTZ, J.B., NICOLAISEN, E.M., CHRISTENSEN, P.E. (1979) Lymphocytosis-promoting factor for *B. pertussis*: A mitogen for human T-lymphocytes. In *International Symposium of Pertussis* (ed. by C.R. Manclark and J.C. Hill). Publ. No. (NIH) 79-1830, pp. 156, NIH, Bethesda, Maryland.

CHABY, R., AYME, G., CAROFF, M., DONIKIAN, R., HAEFFNER-CAVAILLON, N., LEDUR, A., MOREAU, M., MYNARD, M-C., ROUMIANTZEFF, M. & SZABO, L. (1979) Structural features and separation of some of the biological activities of the *B. pertussis* endotoxin by chemical fractionation. In *International Symposium of Pertussis* (ed. by Manclark, C.R. and Hill, J.C.). Publ. No. (NIH) 79-1830, pp. 195, NIH, Bethesda, Maryland.

DRESSER, D.W. & PHILLIPS, J.M. (1973) The cellular targets for the action of adjuvants: T-adjuvants and B-adjuvants. *Ciba Foundation Symp.* **18,** 3.

GEPTS, W. & DEMEY, J. (1978) Islet cell survival determined by morphology—an immunochemical study of the islets of Langerhans in juvenile diabetes mellitus. *Diabetes*, **27,** 251.

HO, M-K., KONG, A.S. & MORSE, S.I. (1980) The *in vitro* effect of *Bordetella pertussis* lymphocytosis-promoting factor on murine lymphocytes. V. Modulation of T-cell proliferation by helper and suppressor lymphocytes. *J. Immunol.* **24,** 36.

HUANG, S-W. & MACLAREN, N.K. (1978) Antibodies to nucleic acids in juvenile-onset diabetes. *Diabetes*, **27,** 1105.

HUANG, S-W. & TAYLOR, G.E. (1981) Immune insulitis and antibodies to nucleic acids induced with streptozotocin in mice. *Clin. exp. Immunol.* **43,** 425.

KATADA, T. & UI, M. (1977) Spontaneous recovery from streptozotocin induced diabetes in rats pretreated with pertussis vaccine or hydrocortisone. *Diabetologia*, **13,** 521.

LIKE, A.A. & ROSSINI, A.A. (1976) Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. *Science*, **193,** 415.

LIKE, A.A., APPEL, M.C., WILLIAM, R.M. & ROSSINI,

A.A. (1978) Morphologic and physiologic studies. *Lab. Invest.* **38,** 470.

MACLAREN, N.K., NEWFELD, M., MCLAUGHLIN, J.V. & TAYLOR, G.E. (1980) Androgen sensitization of streptozotocin-induced diabetes in mice. *Diabetes*, **29,** 710.

MARK, V. (1959) An improved glucose-oxidase method for determining blood, CSF and urine glucose levels. *Clin. Chem. Acta*, **4,** 395.

MUNOZ, J.J. & BERGMAN, R.K. (1979) Biological activities of *B. pertussis*. In *International Symposium of Pertussis* (ed. by Manclark, C.R. and Hill, J.C.). Publ. No. (NIH) 79-1830, pp. 143, NIH, Bethesda, Maryland.

ROSSINI, A.A., WILLIAM, R.M., APPEL, M.C. & LIKE, A.A. (1978) Complete protection from low-dose streptozotocin-induced diabetes in mice. *Nature*, **276,** 182.

SUMI, T. & UI, M. (1975) Potentiation of the adrenergic beta-receptor-mediated insulin secretion in pertussis-sensitized rats. *Endocrinology*, **97,** 352.

UI, M., MIZUSHIMA, Y., KANBAYASHI, Y. & YAJIMA, M. (1977) Extraction and purification of islet activating protein from the culture supernatant fluids of *Bordetella pertussis*. *J. Jap. Diabetic Soc.* **20,** (Suppl.), 22.